THE POLE DISC OF CHRYSOMELID EGGS.

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Hegner (09*b*)[1] has described a disc-shaped mass of darkly staining granules at the posterior end of freshly laid eggs of two genera of beetles, *Calligrapha and Leptinotarsa.* In earlier publications (Hegner, 08, 09*a*)[2] these granules are spoken of as "germ cell determinants" in the sense that they fix the character of the sex cells. The use of the word determinant is open to criticism inasmuch as the term implies the attribute of certain potentialities that these granules have not been shown to possess. In the complete account of the early history of the germ cells (Hegner, 09*b*)[1] the word *determinant* does not appear, and the conclusion regarding their significance is summed up in the statement, that the "pole-disc" is "intimately associated with the development of the pole cells" (p. 288).

In view of the experiments of Lyon, Lillie, Morgan and others, which center about the question of the rôle of preformed materials in the egg as *versus* a predetermined method of action as the essential factor in embryonic development, it is important to know something of the nature and origin of the granules of the pole disc. The data derived from the experiments of these investigators tend to indicate that early developmental phenomena can take place even though the original configuration of the ground substance be radically changed. The formative processes do not depend on the materials displaced by centrifuging, but are bound up in the organization of the ground substance of the egg protoplasm.

Hegner did not determine the origin of the pole disc, but is inclined to believe that it is composed of particles of chromatin derived from the nucleus. No expulsion of material from the nucleus was actually observed; the conclusion being based

[1] *Journal of Morphology*, XX., 2, 1909.

[2] BIOLOGICAL BULLETIN, XVI., 1, 1908; *Journ. Exp. Zoöl.*, VI., 4, 1909.

mainly on staining reactions and the results of Blochmann (86),[1] Stuhlmann (86),[2] and others who have described in various species of hymenoptera a budding of the nucleus of the ovocyte resulting in the formation of many small "nuclei" (Nebenkerne) each containing small dark staining granules. The work of Sylvestri (08)[3] is also cited as supporting a nuclear origin of the pole disc.

In connection with some work embodied in a paper now in press, I have had occasion to study the process of nutrition in the egg of *Leptinotarsa signaticollis*, a chrysomelid beetle closely related to *L. decemlineata*, one of the species studied by Hegner. In this form the nutritive material consists of a granular mass secreted by the nurse cells of the ovariole. This material enters the egg through the egg-string which is a protoplasmic process of the egg terminating in the groove-like spaces between the nurse cells. In safranin-lichtgrün preparations these granules stain as intensely with the basic dye as the granules of the pole disc.

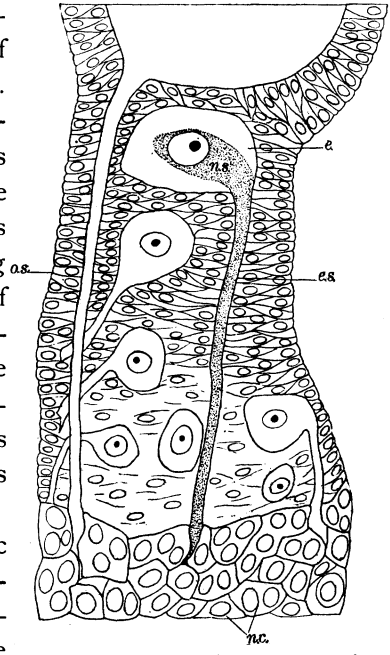

FIG. 1. Longitudinal section of an ovariole showing ovocytes at the beginning of the growth period. The nutritive stream (*n.s.*) is represented in only one of the eggs (*e*). *e.s.*, egg string; *n.c.*, nurse cells; *o.s.*, ovariole stalk.

Fig. 1 is a semi-diagrammatic drawing representing a longitudinal section through an ovary containing developing eggs. The eggs which in an earlier stage are massed together in the proximal region of the terminal chamber of the ovariole, gradually become arranged in a linear series in the ovariole stalk, the cells of which form the egg-follicles. The egg-strings elongate as the eggs move down into the follicles,

[1] *Festschr. Nat. Med. Vereins. Heidelberg.*

[2] *Ber. d. Nat. Ges. Freiberg*, I.

[3] *Bolle. Lab. zool. gen. e agr. della R. Scuola Superiore d'Agricoltura di Portici*, III., 1908.

and pass back from the proximal end of the egg (which represents the anterior end of the future embryo) to the nurse cells.

When the egg is at the stage of development shown at *e*, the food material passes in a broad stream toward the nucleus beyond which it extends for a short distance. A little later (Fig. 2) the form of the nutritive stream changes so that now it encloses a central area of newly formed yolk. The nucleus has shifted its position to the distal (posterior) end of the egg, where it appears as a rounded body composed of an acid staining ground substance in which a number of basic staining nucleoli are embedded.

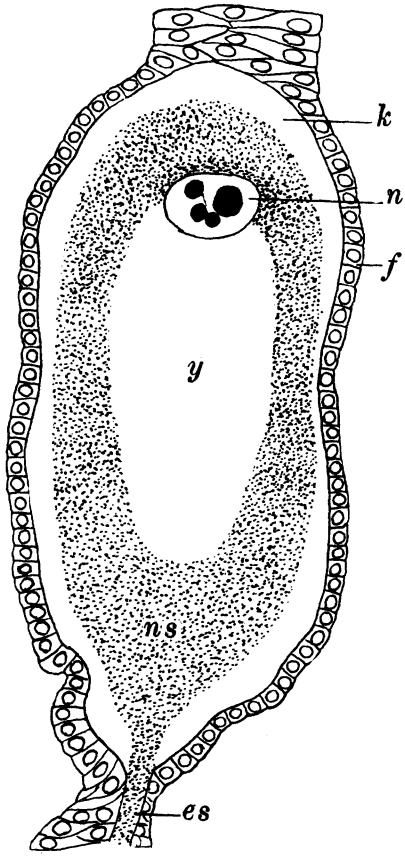

FIG. 2. Longitudinal section of a half-mature ovocyte. *e.s.*, egg string; *f.*, egg follicle, formed by the cells of the ovariole stalk; *k.*, Keimhaut; *n.*, nucleus; *n.s.*, nutritive stream.

When a portion of the cytoplasm containing the food stream is examined at a high magnification, granules of different sizes are seen distributed on an irregular reticulum (Fig. 3). The granules at their point of entrance into the egg as well as those found in the cytoplasm of the nurse cells are of a uniformly small size; while inside of the egg they are of various sizes, due either to coalescence of the small granules or to an actual growth of individual granules. These granules now pass through an interesting cycle of stages. When they have attained their full size they stand out from the reticulum and project into the interreticular spaces (Fig. 4, *a*). They now divide (Fig. 4, *b*). This division is very exact and divides each spherule into two bean-shaped halves.

A series of divisions now ensues until a number of bodies, like chromosomes in appearance, is produced in a single space (Fig. 4, *c*). After about four or five divisions have taken place, they begin to lose their affinity for the basic stain and also their regularity of outline (Fig. 4, *d*), and in the next stage (Fig. 4, *e*) they are seen to have fragmented into numerous irregular bodies that stain with the acid dye. Still later (Fig. 4, *f*) the space is practically filled with a finely granular acid staining yolk mass.

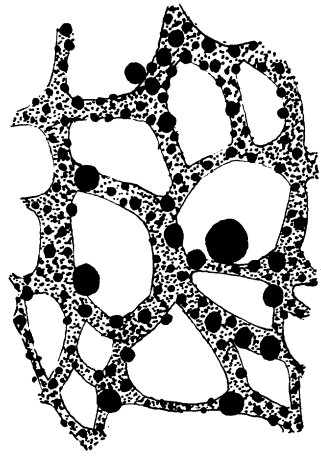

FIG. 3. Section of the cytoplasm of the growing ovocyte in the region of the nutritive stream. × 2,000.

The process of yolk formation, therefore, consists in the transformation of the basic staining granules of the food stream into an acid staining yolk which fills the meshes of the cytoplasmic reticulum of the mature egg. In this transformation the cytoplasm as well as the nucleus is undoubtedly involved. The yolk is first laid down in the center of the egg, but as the granules spread outward as well as inward from the nutritive stream it is not long before yolk is found on either side of the food stream.

The pole disc does not appear until the egg is nearly mature, and a study of successive stages shows that it is composed of granules of the food stream that have accumulated at the posterior end of the egg (Fig. 5). These granules are much larger than those of the Keimhaut (the peripheral layer of cytoplasm surrounding the yolk in the mature egg) and stain intensely with the basic stain. This staining reaction might suggest that the granules are of nuclear origin; but while the nucleus at this time is far from inactive, with an interchange of materials between it and the cytoplasm probably taking place, I have never been able to observe an actual emigration of granules from the nucleus into the cytoplasm.

Union of the germ nuclei in fertilization and the early cleav-

ages take place approximately in the center of the egg. As cleavage proceeds there occurs a separation of the nuclei into two groups, one of which moves outward into the peripheral cytoplasm to form the nuclei of the "Keimhautblastem," while the

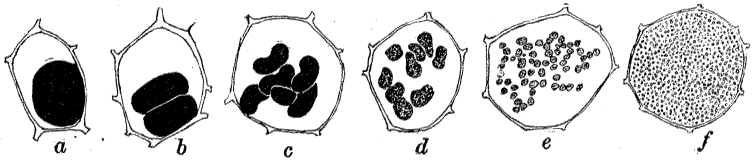

FIG. 4. Six stages in the division of a spherule of the nutritive stream in the process of yolk formation. × 1,200.

other (the vitellophags) remains scattered through the yolk. According to Hegner a definite number of nuclei reaching the posterior part of the egg do not remain in the peripheral cytoplasm, but collect about them a number of granules from the pole disc and continue their migration until they are entirely separated from the blastoderm. These nuclei with their accumulations of cytoplasm constitute the primordial germ cells.

Fig. 6 represents a section taken through the region of the pole disc shortly after the pole cells have reached the periphery. It will be noticed that the granules of the pole disc are much finer than in the earlier stage shown in Fig. 5. The granules in the

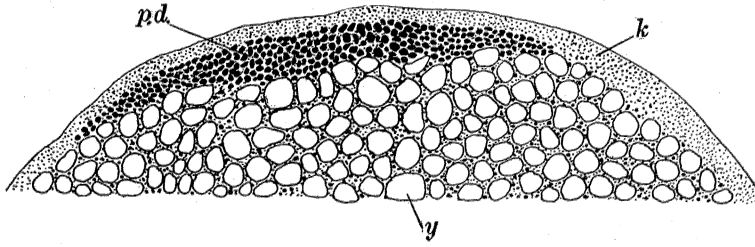

FIG. 5. Longitudinal section of the posterior end of a mature ovum. × 1,000 *p.d.*, pole disc; *k.*, Keimhaut; *y.*, yolk.

cells are distributed on a reticulum and stain intensely with the basic dye, but no more so than the granules that are found in the cytoplasm of the blastema cells. In one case the granules are derived from the pole disc, while in the other they come direct from the food stream. The original source in both cases is the material secreted by the nurse cells.

Does the fact that the granules of the pole disc are derived from the food stream in any way modify our conception of their significance? Were they derived from the chromatin of the nucleus, the fact might be regarded as supporting their hypothetical rôle of germ cell determinants, inasmuch as it would be in accord with the view that the chromatin plays a primary part in directing and perhaps determining the activity of the cell. On

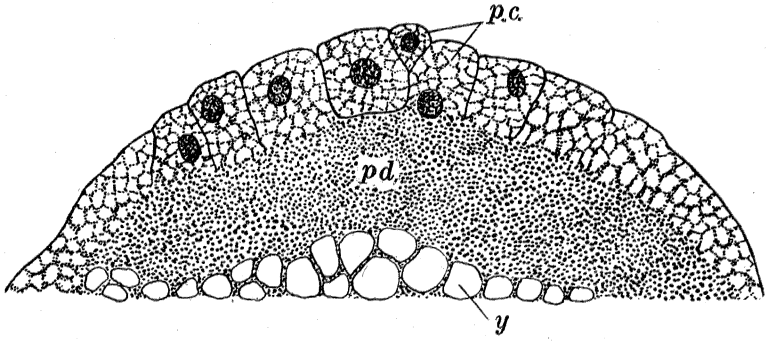

FIG. 6. Longitudinal section of the posterior end of the early embryo. × 600. *p.c.*, pole cells; *p.d.*, pole disc; *y.*, yolk.

the other hand, if they represent so much food material, their significance as germ cell determinants appears in a different light.

The granules of the food stream are derived from the cytoplasmic granules of the nurse cells, which are germ cells that have lost the function of reproduction and that have taken up secondarily the function of secreting material for the growth and development of the ova. In early stages the nurse cells do not differ in any regard from their sister cells destined to become ovocytes. In my paper referred to above I have described what appear to be some of the factors in the differentiation of the primordial germ cells into nurse cells and sex cells, and shall not enter into the matter here.

At the time of this differentiation the nurse cells pass through a period of amitotic divisions, at which time the usual staining reactions of nucleus and cytoplasm become reversed, *i. e.*, the nucleus stains with the acid dye, while the cytoplasm, including the granules which later form the nutritive stream, stains with the basic dye.

The granules may therefore be of the nature of chromatin, and actually represent the chromatin of the nurse cells; for the chromosomes never appear in these cells after they have divided amitotically. But even though this be the case a nuclear origin for these granules loses any special germinal significance when we remember that the latter develop into yolk as well as pole disc, and are therefore just as much "yolk determinants" as "germ cell determinants."

It would appear that a fact of far greater importance in the determining of the germ cells lies in the migration of the pole cells to a position outside of the yolk. This migration isolates these cells and places them in an environment that is entirely unique. It is rather difficult to understand how the absorption of pole disc granules could be the cause of this. The fact that the pole disc occupies a position between the pole cells and the yolk gives a considerable foundation for regarding it as a source of nutrition for these cells.

Hegner states that the granules of the pole disc are absorbed by the pole cells in passing through; after which no further importance is attached to the pole disc. However the granules are not all taken up by the cells in their migration and the greater part of them remains behind after the cells have passed through (Fig. 6).

If then the pole disc represents a part of the nutritive stream of the ovum that has not been transformed into ordinary yolk, but instead has been reserved to supply the pole cells, the conclusion presents itself that the latter as a result of this special kind of nutrition undergo a peculiar method of metabolism which differentiates them from the somatic cells. When these cells are ready to immigrate into the embryo through the pole-cell canal the differentiating factor has already acted and the germ cells are readily distinguished by certain morphological peculiarities from the somatic cells.

It would be highly interesting to know whether or not the germ cells will develop in the absence of the pole disc. The experiments devised by Hegner to test this point have been negative in results, although they do show that the egg may have its contents profoundly disturbed without preventing the production

of a normal embryo. When the granules of the pole disc are displaced by the centrifuge, they are said to move *en masse*, which indicates that they form a structural feature of considerable rigidity.

While more decisive experiments are needed to clear up this point, the evidence at the present time from the morphological side shows that the granules of the pole disc consist of particles derived from the food stream of the ovum that form an accumulation in the protoplasm in its posterior part.